American Journal of

Biochemistry and Molecular Biology

ISSN 2150-4210

Academic Journals Inc.

www.academicjournals.com

American Journal of Biochemistry and Molecular Biology 2 (1): 40-47, 2012
ISSN 2150-4210 / DOI: 10.3923/ajbmb.2012.40.47

Comparative Efficacy of Some Plant Extracts against Fungal Deterioration of Stucco Ornaments in the Mihrab of Mostafa Pasha Ribate, Cairo, Egypt

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ABSTRACT

The demand for medicinal plants is expanding rapidly where plants are now recognized as a safe, efficient and in expensive fungicides for treatment of stucco ornaments. In this study, three different plants extracts *Anethum graveolens*, *Cymbopogon citrates* and *Juniperus oxycedrus* occurring naturally in the plant essentials were evaluated to inhibit the fungal growth of the stucco ornaments in the Ribate of Mostafa Pasha (Azdomor Al Salehy) that belonged to the Ayyubid period. Three fungal species namely: *Fusarium oxysporum*, *Aspergillus niger*, *Alternaria alternate* were isolated from different sites on the tested stucco ornaments. The toxicity of the three plant extracts concentrations against fungal growth revealed that *Cymbopogon citrates* at 100% recorded the highest significant inhibition zones (6.92, 8.0 and 5.19 cm) in all tested fungal species (*A. niger, F. oxysporium* and *A. alternate*), respectively. It is the most effective one in stopping fungal deterioration followed by *Anethum graveolens* fungicide which only retarded fungal growth while *Juniperus oxycedrus* was least effective.

Key words: Stucco ornaments, fungi, mihrab, ribate, *Anethum graveolens*, *Cymbopogon citrates*, *Juniperus oxycedrus*

INTRODUCTION

Growth of microorganisms on decorative stucco cause aesthetic and structural damage. Stucco may be colonized by communities of organisms that interact with the substrate at different stages (Ciferri, 1999). It must be pointed out that the biodeterioration of a given artifact commonly results from the complex interaction established by the microorganisms co-existing simultaneously (Warscheid and Braams, 2000). Suitable temperatures and moisture content combined with rich nutrients in the medieval substrate create suitable conditions for reproduction of quiescent fungal spores. Humidity and heat permit for hyphae germination that penetrate the substrate, with mechanically destructive effects (McNamara and Mitchell, 2005).

As colonization proceeds, the smooth surface of the painting is modified: Pigments initially compact and resistant to attack by various agents become rough and bioreactive (Giullitte, 1995). These events give rise to exfoliation, cracking and loss of the coloured patina (Ciferri, 1999). Certain deteriogenic fungi change cell surface molecules, causing genetic recombinations (Vestrepen *et al.*, 2004). Their metabolic products cause further chemical damage (Angell and Chamberlain, 1991). The capacity of fungi to dissolve carbonates depends on available carbon sources, such as oxalic and citric acids which may mobilize cations with chelating activity (Hirsch *et al.*, 1995). Fungi are an important constituent of microbial endolithic assemblages in moist ecosystems (Golubic *et al.*, 2005). Growth and viability of fungi on building materials under moistening and drying conditions were investigated by Pasanen *et al.* (2000).

Several preventive and remedial methods have been used in tropical environments for control and eradication of microorganisms on stucco monuments. Remedial methods are aimed at the direct elimination by chemical treatments to eliminate and control the growth of biodeteriogens (Curri, 1978; Geweely and Afifi, 2011). Fungal damage on archaeological building materials and their control measures in some museums was studied, where *Aspergillus flavus, A. versicolor, Aureobasidium* sp. and *Penicillium frequentans* was recorded by Cepero *et al.* (1992) and *Cladosporium* sp. was detected by Warscheid *et al.* (1992) and Kumar and Sharma (1992) evaluates biocides for preservation of architectural building materials.

Natural plant materials are important sources of some of the widely used plant extracts (Salehi Sourmaghi *et al.*, 2006; Thatoi *et al.*, 2008; Ajibesin *et al.*, 2008; Keymanesh *et al.*, 2009). Flowers, leaves, roots and other parts are finally ground and used or the toxic principals are extracted and utilized (Mondal and Khalequzzaman, 2010; Isman, 2006; Benamar *et al.*, 2010). Several investigations have been directed towards the antimicrobial activity of essential oils: (Adebajo *et al.*, 1989; Gangrade *et al.*, 1991; Kishore *et al.*, 1980; Mishra *et al.*, 1990; Skukla and Tripathi, 1987). Leaf extract of lemongrass was significantly inhibited the radial growth of *Aspergillus niger, Fusarium moniliforme* (Zaman *et al.*, 1997; ChemMatters, 2004).

The aim of the present study was to investigate the fungal deterioration phenomena of stucco ornaments in Ribate of Mostafa Pasha, Cairo, Egypt which suffer from severe fungal deterioration. Hence, preservation of this material from fungal biodeterioration by using safe fungicide was a national goal in this work.

MATERIALS AND METHODS

Evaluation of the possibility of using plant extracts especially botanical fungicide in preservation of the tested stucco was carried out. Sensitivity of isolated fungal species against three different plant extracts was tested.

Source of isolation and field observation: Ribate of Mostafa Pasha has many stucco decoration in the mihrabs. These decorations suffered from different forms of deterioration grades as discoloration black to dark green due to fungal colonies. It is clear and scattered at different parts particularly in the damped parts. Many cracks and lost parts at different altitudes in the stucco decorations are observed.

Isolation and identification of fungi from tested sample: Samples from markedly damaged stucco surfaces were collected. Swabbing with sterile cotton swabs and scalpel from markedly damaged surfaces with visible colonies of microscopic fungi was carried out. In the laboratory, swab samples were shaken mechanically for 10 min in 10 mL sterile distilled water and 1 mL aliquots of the resulting suspensions used to prepare spread plates on Czapeck's Dox agar in order to isolate as wide a range of microfungi as possible. Plates were incubated in the dark at laboratory temperature (25°C) for 7 days and the microscopic fungi were identified using the diagnostic keys of Booth (1977), Gilman (1957), Barnett and Hunter (1972) and Moubasher (1993).

Plant extracts inhibition zone: Control of fungal growth on stucco decoration from the mihrab of Mostafa Pasha Ribate by plant extracts inhibition zone was performed. Three plant extracts were used in protecting stucco from biodeterioration namely: *Anethum graveolens, Cymbopogon citrates, Juniperus oxycedrus*. Aliquots of about 15 mL of Czapeck's Dox agar were dispersed into sterile

Table 1: Surveys of genera and species of fungi isolated from stucco samples

Fungal species	Count of species	Frequency of occurrence
Aspergillus niger	16	High
Fusarium oxysporum	9	Moderate
Alternaria alternata	5	Low
Total count	30	
No. of species	3	

petri dishes. Each dish was fissured by using sterilized cork borer (1 cm diameter hole) at its center and 1 mL of each plant extract was added.

Each plant extract was used in five concentrations 10, 30, 50, 70 and 100% and was put in each hole and each petri dish was inoculated with tested fungi which cut from the colony margin of 2-4 day old cultures. The plates were incubated for 24 h intervals, after which the diameters of inhibition zone (in cm) were measured.

RESULTS AND DISCUSSION

Frequency of occurrence of fungal species isolated from Stucco Ornaments in the Mihrab of Mostafa Pasha Ribate: Three deteriorating fungal species were isolated from different sites on stucco. Genus *Aspergillus* contributed the broadest spectra where *A. niger* showed highest occurrence as it occured in the all tested sites on stucco. This result agree with Abdel-Hamid and Ouf (1990) who recorded that *Aspergillus* genus was the broadest deteriorating factor causing damage of materials and also Bisht (1995) said that the most common bio-deteriorating fungal species were *Aspergillus, Cladosporium, Alternaria* and *Penicillium*. On the other hand, *Fusarium oxysporum* was isolated in moderate occurrence and *Alternaria alternata* was low occurrence (Table 1).

It is distinctly possible that the minerals may have been contaminated by airborne spores. Hyvarinen *et al.* (2004) stated that mold growth of *Aspergillus* spp. son moistened damage building materials with numbers was between 10 and 10^8 cfu g^{-1} and associated with adverse health effects. Eight microfungi on 21 different types of building material was investigated and only *Penicillium*, *Aspergillus* were a dominant species (Nielsen *et al.*, 2004). The record of fungal occurrence on the building material points to the presence of dung (Van Geel *et al.*, 2003). Fungi were isolated from the damaged building materials could use organic substances of restoration material as nutrient sources for growth on inorganic building materials, it is in fact the interrelationships which play the main role within microbial communities of this type (Karpovich-Tate and Rebrikova, 2002).

Comparative assessment of microbiological deterioration of constructional material which is part of the cultural heritage was achieved by Herrera *et al.* (2004).

Plant extracts sensitivity: Three plant extracts namely: *Anethum graveolens*, *Cymbopogon citratus* and *Juniperus oxycedrus* have been applied on the isolated mould colonization from deteriorated stucco decorations. The data revealed that a significant decrease in the growth of all isolated fungal species with progressive increasing in the concentration of the all three tested plant extracts. The lowest concentrations (10-30%) of the tested *A. graveolens* and *J. oxycedrus* extracts have no effect on the growth of three tested fungal species.

The most resistant tested fungal species was *Alternaria alternata*, where the lowest inhibition zone (2.1 cm) was occurred with 100% concentration of *A. graveolens* extract, on the other hand

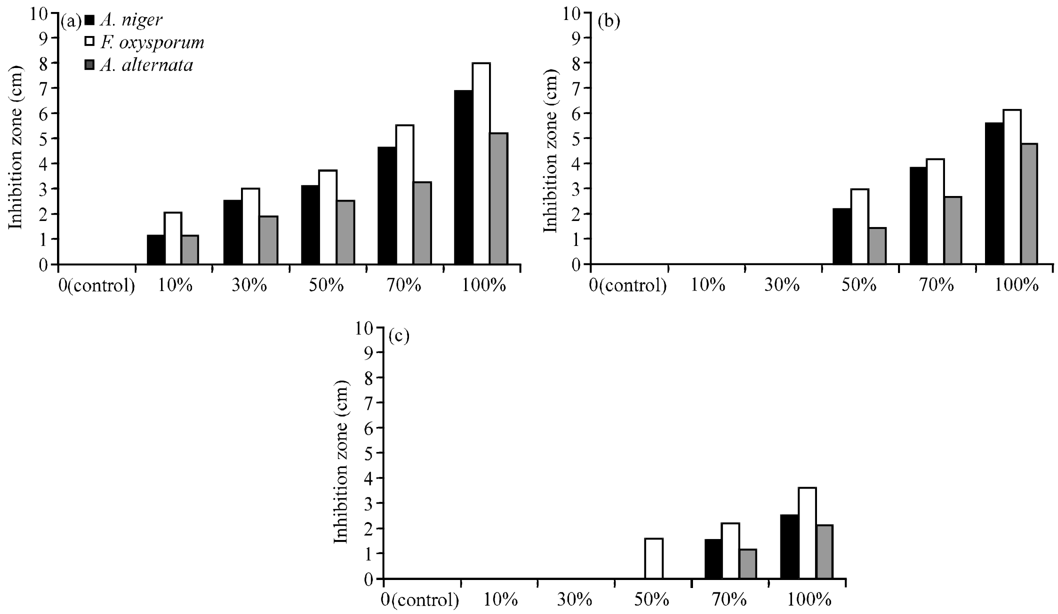

Fig. 1 (a-c): Effect of different concentration of (a) *Cymbopogon citrates,* (b) *Juniperus oxycedrus* and (c) *Anethum graveolens*, extracts on the growth of the three isolated dateriorated fungal species, LSD at 0.05 = 0.50, 1.54 and 2.11, respectively

Fusarium moniliform was considered the most sensitive tested fungal species, where the maximum significant inhibition zone (8.0 cm) was showed with *C. citratus* at 100% concentration (Fig. 1a, b). This may refer to the spores of *Alternaria* are pigmented and more resistant to the inhibitory effect of the tested plant extracts where certain correlations between morphology of the spores and their susceptibility to inhibitor were indicated. This finding agree with that obtained by Geweely (2009) and Hibben and Stotzky (1969) who stated that the spores were more sensitive to inhibition are relatively hyaline, while the most resistant spores were large and pigmented.

Fungicides sensitivity on the isolated fungal species (fungicides inhibition zone): The obtained data revealed that *Cymbopogon citrates* at 100% recorded the highest significant inhibition zones (6.92, 8.0 and 5.19 cm) in all tested fungal species (*A. niger, F. oxysporium* and *A. alternata,* respectively) Fig. 1a. The obtained data was agree with that obtained by Fiori *et al.* (2000) who stated that the plant species responsible for the greatest inhibition of the mycelial growth of microorganisms was *C. citratus.* The highest inhibitory effect of *Cymbopogon citratus* may be referred to the presence of antifungal protein in the extract. Linthorst and Van Loon (1991) stated that low molecular weight proteins or peptides in the plant extract have antifungal activities. They were believed to be involved in a defense mechanism against pathogenic fungi by inhibiting microbial growth through binding to chitin or increasing the permeability of the microbial membranes or cell wall. According to the explanation of Farag *et al.* (1989), it appears that there is a relationship between the chemical structure of the most abundant compounds in the plant extract and the antimicrobial activity. Fiori *et al.* (2000) indicated that the plant species responsible for the greatest inhibition of the mycelial growth of microorganisms was *Cymbopogon citratus.* Tzortzakis and Economakis (2007) tested the antifungal activity of Cymbopogon citratus oil against *Aspergillus niger.* According to the explanation of Farag *et al.* (1989), it appears that there is a relationship between the chemical structure of the most abundant compounds in the essential oils and the anti-microbial activity. The data in Fig. 1b showed that applying of 100% *J. oxycedrus* on the tested fungi gave the moderate inhibitory effect. On the other hand *A. graveolens* had low inhibitory effect against all tested fungal species (Fig. 1c). Building materials are shown to be deteriorated by a variety of fungi and the control was achieved by Nalli *et al.* (2006), Schieweck *et al.* (2005), Do *et al.* (2005) and Clausen and Vina (2005). Tzortzakis and Economakis (2007) tested the antifungal activity of *Cymbopogon citratus* oil against *Aspergillus niger*. Differences in sensitivity to fungicide also existed between strains of the same species (Dannenhauer *et al.*, 1983).

CONCLUSION

Mostafa Pasha Ribate belonged to the Ayyubid period has many stucco ornaments in the two mihrabs. These decorations have many colord spots due to some fungal deterioration. Three of plant extracts were examined against the deteriorated tested fungal species. *Cymbopogon citratus* was the best one for controlling all tested fungal species. Treatment of the stucco ornaments of the mihrab must be carried out by applying *Cymbopogon citrates* extract, where the highest inhibition zones were showed in *A. niger*, *F. oxysporium* and *A. alternata*, respectively. It was the most preferable inhibitor to prevent fungal growth instead of using chemicals fungicides to avoid the unfavorable side effect of chemicals on the properties of archeological stone and public health and environment.

ACKNOWLEDGMENT

I would like to express my deepest thanks to Dr. Neveen S.I. Geweely, Associate professor of microbiology, Department of Botany, Faculty of Science, Cairo University her assistance and helpful discussions.

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